

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

United States Patent and Trademark
Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 20 May 1999 (20.05.99)	
International application No. PCT/GB98/02904	Applicant's or agent's file reference WN/NV/WCM.63
International filing date (day/month/year) 29 September 1998 (29.09.98)	Priority date (day/month/year) 30 September 1997 (30.09.97)
Applicant LUDGATE, Marian, Elizabeth	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

01 April 1999 (01.04.99)

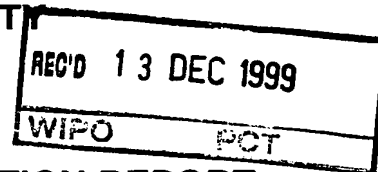
☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Lazar Joseph Panakal Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY

PCT



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference WN/NV/WCM.63/PCT	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB98/02904	International filing date (day/month/year) 29/09/1998	Priority date (day/month/year) 30/09/1997
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant UNIVERSITY OF WALES COLLEGE OF MEDICINE et al.		



1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

 These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 01/04/1999	Date of completion of this report 0 8. 12. 99
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Hinchliffe, P Telephone No. +49 89 2399 8431 

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/02904

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-14 as originally filed

Claims, No.:

1-43 as published

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-43
	No:	Claims
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-43
Industrial applicability (IA)	Yes:	Claims 1-43
	No:	Claims

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB98/02904

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

SECTION V

1. The subject matter of claim 33 (and its dependent claims) is novel according to the requirement of Article 33(2) PCT. D1 (as cited in the I.S.R.) discloses a reporter construct which contains the CAT gene (a reactant) and a cAMP response element (TGACGCGTCA- see fig.5). However D1 does not disclose the use of this construct in the detection of TSH or TSH autoantibodies.
2. The subject matter of claims 36-38 and 39 is novel according to the requirement of Article 33(2) PCT. D1 discloses that the constructs, shown in fig.5, were transfected into JEG-3 cells. However it has been shown that this cell type does not express the TSH receptor, and thus the said claims are novel.
3. The subject matter of claims 1 to 43 does not fulfill the requirements of Article 33(3) PCT because it does not appear to involve an inventive step.
The assay kit and method claimed are based upon assaying, indirectly, the effect of an effector molecule (TSH or TSH-R autoantibodies) on the TSH receptor. However D3 also discloses an indirect assay for TSH-R autoantibodies in which cell lines stably transfected with the TSH receptor are assayed for cAMP, which levels are controlled by the TSH receptor. The difference between the prior art method and the currently claimed one is therefore in how the cAMP is measured. The applicants allege that the selection of a reporter system stimulated by cAMP is inventive insofar as it provides advantages over the method of assay of D3 (see description p. 3). Whereas this is true, the choice of such a reporter system for this sort of assay is known from D2. More interestingly, D2 notes that the receptors are linked to their specific effector functions via nucleotide regulatory elements and that an assay of cAMP, using a reporter system linked to cAMP responsive elements, was used to detect the activity of such receptors (see introduction). In addition the abstract of D2 notes that:
"The non-isotopic assay can be performed in microtitre plate format and is far less work intensive than the determination of adenyl cyclase activity by direct cAMP measurement". D2 also notes, on p.91, that direct assays based upon other reporter constructs (such as CAT) produce similar results to those produced with the luciferase system it discloses, however these CAT based systems do not allow themselves to be as easily automated as the luciferase system. Such a disclosure

does not prejudice the adaption of the system of D2 to the assay of D3 as alleged by the applicant. Consequently the skilled person is provided with a made to measure reporter system (D2) for the intermediate signalling agent (cAMP) and an assay for TSH-R wherein the cAMP detection is complex. It therefore appears to be completely obvious for a skilled person, when faced with the problem of simplifying the assay of D3, to apply the system of D2 and arrive at the subject matter of the current claims without recourse to inventive skill.

SECTION VII

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D2 are not mentioned in the description, nor are these documents identified therein.

SECTION VIII

1. Claim 1 does not fulfill the requirement of Rule 6.3b(i) because essential technical details are missing from the said claim. In particular the claim refers to an assay but fails to recount how the assay should be carried out. The objection could easily be overcome by adding the steps given in claims 2 and 3.
2. Claim 14 is entirely unclear, contrary to Art. 6 PCT, because it attempts to define a kit by referring to a method. Therefore the exact constituents of the kit are not defined in this claim.
3. Claims 18-24 are unclear contrary to the requirements of Art. 6 PCT because the plasmid and DNAs referred to therein mean nothing to the skilled person unless it can be shown they are known and available to the public. Furthermore claim 25 (and dependent claims) refers to a cell line identified herein, however, the said cell line is not identified in the claims as it should be. Therefore claim 25 is also unclear contrary to the requirements of Article 6 PCT.
4. Claim 30 is unclear contrary to the requirements of Art. 6 PCT because there is no association between a disease and a test kit. The use of a test kit for the detection of a disease or condition associated with autoimmune thyroid disease appears to

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB98/02904

be what was intended.

5. Claim 32 is unclear contrary to the requirements of Art. 6 PCT because the modifications made to the TSH-R sequence are both undefined and not encompassed by the description.

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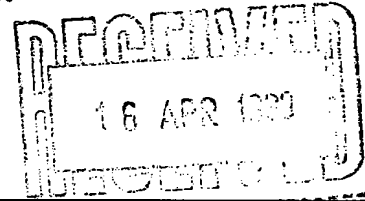
**NOTICE INFORMING THE APPLICANT OF THE
COMMUNICATION OF THE INTERNATIONAL
APPLICATION TO THE DESIGNATED OFFICES**

(PCT Rule 47.1(c), first sentence)

From the INTERNATIONAL BUREAU

To:

NEWELL, William, Joseph
Wynne-Jones, Laine and James
Morgan Arcade Chambers
33 St. Mary Street
Cardiff CF1 2AB
ROYAUME-UNI



Date of mailing (day/month/year) 08 April 1999 (08.04.99)		
Applicant's or agent's file reference WN/NV/WCM.63		IMPORTANT NOTICE
International application No. PCT/GB98/02904	International filing date (day/month/year) 29 September 1998 (29.09.98)	Priority date (day/month/year) 30 September 1997 (30.09.97)
Applicant UNIVERSITY OF WALES COLLEGE OF MEDICINE et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
AU,EP,JP,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
CA

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on 08 April 1999 (08.04.99) under No. WO 99/16902

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a **demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

<p>The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No. (41-22) 740.14.35</p>	<p>Authorized officer J. Zahra</p> <p>Telephone No. (41-22) 338.83.38</p>
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PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference WN/NV/WCM. 63	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 98/ 02904	International filing date (day/month/year) 29/09/1998	(Earliest) Priority Date (day/month/year) 30/09/1997
Applicant UNIVERSITY OF WALES COLLEGE OF MEDICINE et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. ☐ **Certain claims were found unsearchable** (see Box I).
2. ☐ **Unity of invention is lacking** (see Box II).
3. ☐ The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
 - ☐ filed with the international application.
 - ☐ furnished by the applicant separately from the international application,
 - ☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
 - ☐ Transcribed by this Authority
4. With regard to the **title**, ☒ the text is approved as submitted by the applicant
☐ the text has been established by this Authority to read as follows:
5. With regard to the **abstract**, ☒ the text is approved as submitted by the applicant
☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.
6. The figure of the **drawings** to be published with the abstract is:
 Figure No. ☐ as suggested by the applicant. ☐ None of the figures.
☐ because the applicant failed to suggest a figure.
☐ because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/02904

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/68 C12N15/12 C12N5/18 C07K14/72

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KRISHNA V. ET AL.,: "Repression of the human glycoprotein hormone α -subunit gene by glucocorticoids:evidence for receptor interactions with limiting transcriptional activators"	32
A	MOL. ENDOCRINOLOGY, vol. 5, no. 1, - January 1991 pages 100-110, XP002089743 see whole doc. esp. figures 4 and 5 --- -/--	8,17

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

12 January 1999

Date of mailing of the international search report

22/01/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Müller, F

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02904

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HIMMLER A ET AL: "FUNCTIONAL TESTING OF HUMAN DOPAMINE D1 AND D5 RECEPTORS EXPRESSED IN STABLE CAMP-RESPONSIVE LUCIFERASE REPORTER CELL LINES" JOURNAL OF RECEPTOR RESEARCH, vol. 13, no. 1/04, 1 January 1993, pages 79-94, XP000471667 see the whole document ---	1-37
Y	LUDGATE M ET AL: "USE OF THE RECOMBINANT HUMAN THYROTROPIN RECEPTOR (TSH-R) EXPRESSED IN MAMMALIAN CELL LINES TO ASSAY TSH-R AUTOANTIBODIES" MOLECULAR AND CELLULAR ENDOCRINOLOGY, vol. 73, 1 January 1990, pages R13-R18, XP000283191 see the whole document ---	1-37
A	PERSANI L. ET AL.,: "Measurement of cAMP accumulation in chinese hamster ovary cells transfected with recombinant human TSH receptor (CHO-R): a new bioassay for human thyrotropin" J. ENDOCRINOL. INVEST., vol. 16, - July 1993 pages 511-519, XP002089744 see the whole document ---	1-37
A	LIBERT F ET AL: "CLONING, SEQUENCING AND EXPRESSION OF THE HUMAN THYROTROPIN (TSH) RECEPTOR EVIDENCE FOR BINDING OF AUTOANTIBODIES" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 165, no. 3, 29 December 1989, pages 1250-1255, XP000083569 see the whole document ---	1-37
T	WATSON P.F. ET AL.,: "A new chemiluminescent assay for the rapid detection of thyroid stimulating antibodies in Graves' disease" CLIN. ENDOCRINOLOGY, vol. 49, - November 1998 pages 577-581, XP002089745 see the whole document -----	

INTERNATIONAL SEARCH REPORT

1st Application No
PCT/GB 98/02904

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/68 C12N15/12 C12N5/18 C07K14/72

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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	-/-	

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

12 January 1999

Date of mailing of the international search report

22/01/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

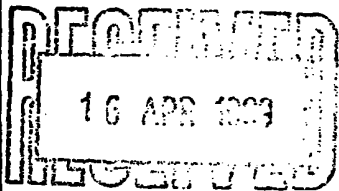
Müller, F

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02904

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HIMMLER A ET AL: "FUNCTIONAL TESTING OF HUMAN DOPAMINE D1 AND D5 RECEPTORS EXPRESSED IN STABLE CAMP-RESPONSIVE LUCIFERASE REPORTER CELL LINES" JOURNAL OF RECEPTOR RESEARCH, vol. 13, no. 1/04, 1 January 1993, pages 79-94, XP000471667 see the whole document	1-37
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A	PERSANI L. ET AL.: "Measurement of cAMP accumulation in chinese hamster ovary cells transfected with recombinant human TSH receptor (CHO-R): a new bioassay for human thyrotropin" J. ENDOCRINOL. INVEST., vol. 16, - July 1993 pages 511-519, XP002089744 see the whole document	1-37
A	LIBERT F ET AL: "CLONING, SEQUENCING AND EXPRESSION OF THE HUMAN THYROTROPIN (TSH) RECEPTOR EVIDENCE FOR BINDING OF AUTOANTIBODIES" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 165, no. 3, 29 December 1989, pages 1250-1255, XP000083569 see the whole document	1-37
T	WATSON P.F. ET AL.: "A new chemiluminescent assay for the rapid detection of thyroid stimulating antibodies in Graves' disease" CLIN. ENDOCRINOLOGY, vol. 49, - November 1998 pages 577-581, XP002089745 see the whole document	





INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification: C12Q 1/68, C12N 15/12, 5/18, C07K 14/72	A1	(11) International Publication Number: WO 99/16902 (43) International Publication Date: 8 April 1999 (08.04.99)
(21) International Application Number: PCT/GB98/02904 (22) International Filing Date: 29 September 1998 (29.09.98) (30) Priority Data: 9720693.2 30 September 1997 (30.09.97) GB (71) Applicant (for all designated States except US): UNIVERSITY OF WALES COLLEGE OF MEDICINE [GB/GB]; Heath Park, Cardiff CF4 4XN (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): LUDGATE, Marian, Elizabeth [GB/GB]; 6 Cyncoed Rise, Cardiff CF2 6SF (GB). (74) Agents: NEWELL, William, Joseph et al.; Wynne-Jones, Laine and James, Morgan Arcade Chambers, 33 St. Mary Street, Cardiff CF1 2AB (GB).		(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.
(54) Title: BIOASSAY FOR THYROID STIMULATING ANTIBODIES (57) Abstract An assay method for TSH-R autoantibodies or TSH comprises contacting a test sample, in the presence or absence of TSH, with cells from a clone expressing human TSH-R transfected with a reporter construct comprising cDNA of both (i) a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein, and (ii) a promoter containing cyclic AMP (cAMP) response elements (CREs), whereby cAMP levels vary with expression of the reactant. Also disclosed are related kits, reporter constructs and related biological material.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
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CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q 1/68, C12N 15/12, 5/18, C07K 14/72	A1	(11) International Publication Number: WO 99/16902 (43) International Publication Date: 8 April 1999 (08.04.99)
(21) International Application Number: PCT/GB98/02904 (22) International Filing Date: 29 September 1998 (29.09.98) (30) Priority Data: 9720693.2 30 September 1997 (30.09.97) GB (71) Applicant (for all designated States except US): UNIVERSITY OF WALES COLLEGE OF MEDICINE [GB/GB]; Heath Park, Cardiff CF4 4XN (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): LUDGATE, Marian, Elizabeth [GB/GB]; 6 Cyncoed Rise, Cardiff CF2 6SF (GB). (74) Agents: NEWELL, William, Joseph et al.; Wynne-Jones, Laine and James, Morgan Arcade Chambers, 33 St. Mary Street, Cardiff CF1 2AB (GB).		(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: BIOASSAY FOR THYROID STIMULATING ANTIBODIES (57) Abstract An assay method for TSH-R autoantibodies or TSH comprises contacting a test sample, in the presence or absence of TSH, with cells from a clone expressing human TSH-R transfected with a reporter construct comprising cDNA of both (i) a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein, and (ii) a promoter containing cyclic AMP (cAMP) response elements (CREs), whereby cAMP levels vary with expression of the reactant. Also disclosed are related kits, reporter constructs and related biological material.		

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BIOASSAY FOR THYROID STIMULATING ANTIBODIES

The present invention relates to an assay for measuring antibodies to the thyrotropin receptor; to an assay kit therefor; and specifically to the use therein of a cell line transfected with α -luciferase cDNA.

5 The thyrotropin receptor (hereinafter 'TSH-R') is known to regulate both the function and proliferation of the thyroid cell, and is stimulated by the hormone thyrotropin (TSH). The TSH-R is also a target for autoantibodies, which inhibit the binding of TSH to the receptor. These autoantibodies either block the action of TSH (TBAb) (*i.e.* TSH antagonists, which act as
10 hypostimulants or inhibitors) or stimulate (hyperstimulate) the thyroid (TSAb) by acting as agonists to TSH.

 Stimulation by TSAb is believed to be a mechanism operating in Graves' disease (GD), whilst inhibition by TBAb is believed to be the case in idiopathic myxoedema. Patients with hyperthyroid Graves' disease produce
15 antibodies which mimic the action of TSH, leading to chronic stimulation of adenylate cyclase; and whereas the autoantibodies in some patients with idiopathic myxoedema are also able to bind to the TSH-R, nevertheless this does not result in an increase in intracellular cyclic adenosine 3',5'-monophosphate (cAMP).

20 Many assays have been developed to measure TSH-R autoantibodies. The most widely used is a radioreceptor assay in which the binding of bovine 125 I-TSH to detergent-solubilised porcine TSH-R is inhibited by immunoglobulins or sera from patients suspected of having TSH-R autoantibodies. The main problems with this assay are that it uses a non-
25 human antigen; and that it measures binding and not biological activity, hence it is not able to distinguish between TSAb and TBAb.

 It is clearly desirable to be able to distinguish between stimulation and inhibition, and therefore attempts have been made to develop a bioassay in which an effect of TSH (or its inhibition by TBAb) or TSAb is measured, such
30 as the increase in cAMP. This may be done in thyroid slices or thyroid cells in

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culture and the greatest sensitivity, defined as the highest percentage of Graves' patients being positive, is achieved when the assay is performed in hypotonic (sodium chloride-free) medium. Both the radioreceptor and the bioassays are hampered by limited availability of biological material. To
5 overcome this, a rat thyroid cell line (FRTL-5) has been developed, but here species differences may still be problematic. The recent cloning and sequencing of the TSH-R give unlimited access to recombinant human TSH-R.

As described by Libert *et al* in Biochem. & Phys. Res. Comm. 165 (3)
10 1250-1255 (1989) [all references herein are herein incorporated in their entirety, together with any cross-references therein], previous cloning of the dog thyrotropin receptor opened the way to molecular characterisation of the human TSH-R *via* isolation of human TSH-R cDNA clones; the analysis of the primary structure of the encoded polypeptide; and evidence that the
15 recombinant molecule binds auto-antibodies found in patients with Graves' disease and idiopathic myxoedema. The dog TSH-R cDNA (a 2.8 kb fragment) was used to hybridise a human thyroid DNA library. Sequencing of the resulting clones gave rise to a 2292 nucleotide residue open reading frame encoding a 744 amino acid polypeptide having 90.3% similarity with the
20 dog TSH-R. Transfection of the coding sequence in the pSVL vector of COS-7 cells allowed confirmation of the protein's ability to bind specifically TSH.

The co-transfection of Chinese hamster ovary (CHO) cells with a pSVL vector containing the coding region of human TSH-R led to the selection of cell lines particularly responsive to TSH or TSA_b in terms of their cAMP
25 accumulation (reported by Perret *et al* in Biochem. & Biophys. Res. Comm. 171 (3) 1044-1050 (1990)). Dose response curves of TSH-mediated cAMP accumulation were reported for clones JP14, JP26 and JP28, and the number of receptors per cell were found to be highest in clones JP14 and JP09.

This work gave rise to the possibility of a bioassay in which cAMP
30 production is measured in CHO cells stably transfected with the human TSH-

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R in the presence of an autoantibody either alone (TSAAb) or in the presence of TSH (TBAAb), (Ludgate *et al* in Molec. & Cell. Endocrin. 73 R13-R18). However, such an assay is not sufficiently robust for routine use since it takes several days to perform, especially in view of the final detection of the generated cAMP by RIA, and requires tissue culture facilities.

Accordingly, the present invention provides an assay method for TSH-R autoantibodies or TSH comprising step:

(a) contacting a test sample, in the presence or absence of TSH, with cells from a clone expressing human TSH-R transfected with a reporter construct comprising cDNA of both a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein, and a promoter containing cyclic AMP (cAMP) response elements (CREs), whereby cAMP levels vary with expression of the reactant.

Preferably, the assay method further comprises step:

(b) adding the corresponding substrate to cells thus contacted.

More preferably, the assay method still further comprises steps:

(c) measuring the response in the cells exposed to the substrate; and
(d) comparing the response from test step (c) with the response from a standard or normal sample which has undergone steps (a) to (c).

The present invention therefore especially provides an assay, for TSH-R auto-antibodies or TSH, comprising:

(a) bringing into contact a test sample with cells from a clone expressing human TSH-R stably transfected with a reporter construct comprising cDNA of both a reactant capable of causing a measurable response when brought into contact with a corresponding substrate and a promoter containing cAMP

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response elements whereby cAMP levels vary with expression of the reactant;

- (b) adding the corresponding substrate to cells thus contacted;
- (c) measuring the response in the cells exposed to substrate; and
- 5 (d) comparing the response from test step (c) with the response from a standard or normal sample which has undergone steps (a) to (c).

In the assay method of the present invention, all reagents used therein may be brought together in one or more steps, such as steps (a) to (d) defined herein. The notation of the steps (a) to (d) is not to be construed as
10 meaning only that each step is carried out sequentially or that each component in the assay must be brought into individual contact with each other. For example, two or more of the steps (a) to (d) may be carried out substantially simultaneously; and/or all reagents used therein may be brought
15 together in one step. The assay method or any combination of the steps therein may be carried out by manual, partly automated or fully automated means.

Suitable reporter constructs (referred to in step (a)) are those in which the enzyme activity results in a colour change, fluorescence change or
20 emission of light. Examples of such enzymes include chloramphenicol acetyl transferase (CAT), Firefly luciferase, Renilla luciferase, β -galactosidase, alkaline phosphatase, horseradish peroxidase or green fluorescent protein.

The cyclic AMP response element (CRE) of the reporter gene could be any promoter sequence or synthetic oligonucleotide which contains the CRE
25 consensus sequence, TGACGTCA, preferably as a number of tandem repeats. A suitable promoter is that for the glycoprotein hormone alpha subunit which contains tandem cAMP response elements, described by Kay *et al* in *Endocrinology* 134 (2) 568-573 (1994). Another example is a construct driving the CAT enzyme which has been described by Chatterjee *et al* in *Molecular Endocrinology* 5(1) 100-109 (1991).
30

However, the assay according to the present invention preferably comprises, in step (a), the use of a luciferase cDNA driven by a promoter containing cAMP response elements; and, in step (b), the use of luciferin; which means that the response measured in steps (c) and (d) is light output
5 from the luciferinised cells. Preferably, the luciferase is Firefly luciferase, although Renilla luciferase or the like would also be suitable.

Most preferably, the reporter construct is α -luciferase, being a luciferase cDNA driven by the promoter for the glycoprotein hormone α subunit, mentioned above. For example, the plasmid pA3luc may be
10 employed, having the glycoprotein hormone α subunit promoter introduced as described by Maxwell *et al* in Biotechniques 7 276-80 (1989). The α -luciferase is therefore 846 base pairs of 5' flanking sequence and 44 base pairs of exon 1 of the glycoprotein hormone α subunit promoter in the plasmid pA3luc. Alternatively, the CRE-containing sequence could be sub-cloned into
15 a commercially-available luciferase reporter system such as the pGEM-luc vector from Promega. A further alternative is to use a plurality of plasmids, such as in the system available from Stratagene (CREB reporting system, no. 219010), which includes plasmids enabling a luciferase response to be measured following an increase in cAMP. Alternatively, inducible plasmids
20 other than CREs could be included.

The cells may be obtained as described in the reference mentioned above by Perret *et al*. Alternatively, the human TSH-R could be subcloned into any eukaryotic expression vector (of which pSVL is an example) available
25 from Stratagene, InVitrogen or the like for transfection into any eukaryotic cell or cell line. For selectivity, the more recently-developed dual vectors that incorporate the antibiotic resistance gene within the same plasmid, such as pcDNAIII (available from InVitrogen) may be used. Otherwise, a separate plasmid for selection may be employed.

Preferably, the cells used in the assay (step (a)) are those identified as
30 from clone JP09 in the above-mentioned reference which have been stably

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transfected with (ie which express) in the order of 10^5 human TSH-R per cell. More preferably, they are co-transfected with both α -luciferase cDNA and a puromycin resistance encoding plasmid such as pSV₂Neo (available from Clontech) to allow selection of assay cells with puromycin. Surviving cells are then tested for luciferase activity in response to TSH. Alternatively, cells which have been transiently transfected with any of these plasmids may be employed.

Therefore, the present invention provides a bioassay comprising human TSH-R expressed in, for example, CHO cells, wherein the improvement comprises (in place of an RIA for cAMP) measuring light output from a luciferase gene driven by a promoter containing CREs. This makes the assay more rapid, enabling the complete evaluation of TSAb, from the point of serum being in contact with the cells through to obtaining data for calculation, within a single working day. Furthermore, this assay can be performed on unfractionated serum, eliminating the need for sample preparation.

Preferably, the cells would be lyophilised (freeze-dried), frozen or comprised in a gel and provided in individual containers with one container being used per assay. Alternatively, the cells could be frozen or incorporated into a gel (such as Matrigel™), for storage.

Another co-transfection may be carried out to provide the assay with a method of correcting for the number of cells seeded in a well during use, in the case where non-lyophilised cells are to be used. Since the Renilla luciferase construct is constitutive and has different substrate requirements from Firefly luciferase, it provides such a method. The same value would be expected from every well whilst differences would reflect varying cell number. An appropriate plasmid for this transfection is the Renilla luciferase plasmid available from Promega, no. E2241. It contains the Herpes simplex virus thymidine kinase promoter upstream from Renilla luciferase, which is thus constitutively expressed.

Furthermore, when using intact rather than lyophilised cells, in order to prevent distortion of the assay by the presence of any TSH present in serum used in the cell culture medium, the serum should be charcoal-stripped at around 24 hours prior to assay.

5 In addition, as with the RIA, TSH responsiveness is reduced in salt-free (ie NaCl-free) conditions. To further enhance assay sensitivity, reagents such as phosphodiesterase inhibitors may be added.

The present invention therefore further provides a reporter construct comprising cDNA of both a reactant, such as an enzyme, capable of causing
10 a measurable response when brought into contact with a corresponding substrate and a promoter containing cAMP response elements, whereby cAMP levels vary with expression of the substrate, in particular wherein the reactant is a luciferase.

Accordingly, the present invention further provides cells from a clone
15 expressing human TSH-R (preferably, stably) transfected with a reporter construct comprising cDNA of both a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein, and a promoter containing CRE; the clone; cDNA or mRNA expressing the (preferably stably) transfected
20 human TSH-R; and human TSH-R (preferably stably) transfected with a reporter construct comprising cDNA of both a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate and a promoter containing CRE.

Preferably, the assay according to this invention is carried out by
25 means of a kit to enable fast and convenient results in a regular medicinal biochemistry laboratory or hospital pathology or diagnostic laboratory. The present invention therefore further provides a kit for carrying out an assay, particularly a bioluminescent assay, of the present invention, which kit comprises:

(a) cells from a clone expressing human TSH-R transfected with a reporter construct comprising cDNA of both (i) a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein, and (ii) a promoter containing cAMP response elements whereby cAMP levels vary with expression of the reactant;

(b) a standard sample for the assay;

(c) medium for culturing and/or reconstituting the cells; and

(d) instructions for carrying out the assay according to the present invention.

The corresponding protein and reagents relating thereto, and means for carrying out the response measurements may also be provided as part of the kit. For example, the kit may further comprise:

(e) buffer for lysing the cells; and/or

(f) buffer for the reporter construct, preferably luciferase buffer; and/or

(g) corresponding substrate, preferably protein, more preferably luciferin, in buffer;

and, optionally, a luminometer.

Alternatively, for example, in the case where luciferase/luciferin are employed in the assay, a separate, commercially-available kit may be employed such as one of those available from the Promega Corporation. These commercially-available kits include no. E1483 wherein the luciferase is Firefly luciferase, and a dual-luciferase system no. E1910, which also employs Renilla luciferase.

The assay method according to the present invention and the kit therefor may be used in association with a condition or disease selected from: autoimmune thyroid disease, non-autoimmune thyroid disease, autoimmunity of non-thyroid origin and polyendocrine disease. For example, they may be used in screening patients selected from: pregnant women, those with

euthyroid eye disease, and those receiving amiodarone and/or lithium. The assay method or kit according to the invention is suitable for measuring TSAb or TBAb, or for measuring autoantibodies to the TSH-R having part of its sequence modified, such as by having one or more of its amino acids replaced or otherwise modified to include tags.

The present invention will now be illustrated with reference to the following non-limiting examples:

Example 1: Preparation of cells for use in luminescent assay for TSH and TSH-R antibodies - Use of Firefly luciferase

Chinese hamster ovary cells (CHO-K1), available from the ATCC number CCL61, were subjected to calcium phosphate co-transfection, using standard protocols (Current Protocols in Molecular Biology, 1996, John Wiley & Sons Inc. section 9.1.4), with pSV₂Neo (available from Clontech) and a eukaryotic expression vector carrying the human TSH-R gene, pSVL-hTSHR (available from G. Vassart, IRIBIIN, Brussels, Belgium), as described by Perret *et al* (1990, *ibid*). The cells were selected with 400 µg/ml geneticin (G418) and cloned by limiting dilution. Clone JP09 was isolated by this method (and is also available from Prof. G. Vassart), which expresses approximately 10⁵ receptors per cell, as assessed in TSH binding experiments (described by Costagliola S, Swillens S, Niccoli P, Dumont J, Vassart G, Ludgate M in J Clin Endocrinol Metab 75 1540 *et seq.* (1992)).

JP09 cells were maintained at 37C in 5% carbon dioxide in air in Ham's F12 medium with 10% foetal calf serum.

Clone JP09 was co-transfected, again using the above-noted standard calcium phosphate method, with a eukaryotic expression vector carrying the puromycin resistance gene (available from, *inter alia*, InVitrogen, Stratagene etc.) and pA3Luc (available from V. Chatterjee, Univ. Cambridge, UK). Cells were selected in puromycin, 2.5 µg/ml and cloned by limiting dilution. Clones were tested for light output (see Example 3) in response to bovine TSH (from Sigma, T8931). Clones giving a good TSH response were tested with a panel

of normal human sera, the selection criterion in this case being a low light output of ≤ 1.5 relative light units.

Example 2: Preparation of cells for use in luminescent assay for TSH and TSH-R antibodies - Use of Firefly and Renilla luciferases

5 The method of Example 1 was followed, but after the second co-transfection involving the puromycin resistance gene, another co-transfection was carried out repeating the standard calcium phosphate method, but instead with a eukaryotic expression vector carrying the hygromycin resistance gene (available from Stratagene) and the R. luciferase plasmid
10 E2241 (available from Promega).

Example 3: Assay for Measurement of (i) TSH bioactivity and (ii) Thyroid Stimulating (TSAB) or (iii) Blocking (TBAB) Antibodies

Culture medium for the cells was RPMI; 10% foetal calf serum (FCS); 1% glutamine; 1% pyruvate; 2% penicillin/streptomycin (and 2.5 μ g/ml
15 puromycin, when amplifying cells for assay). 96 well plates were seeded with 5×10^4 cells prepared according to Example 1 and cultured overnight (approximately 16 hours) at 37°C in a water-saturated incubator. They were then cultured for a second night in medium containing 10% charcoal stripped calf serum instead of FCS (available from Gibco). Basal light output was
20 measured, in triplicate wells, in the presence of 100 μ l of the medium containing 10% charcoal stripped serum. Standards (see below) and test samples were in the form of 10% patient serum to a final volume of 100 μ l of the medium containing 10% charcoal stripped calf serum. Basal, standard and test incubations were for 2-3 hours as above. The final result was
25 expressed as relative light units (RLU) obtained by the ratio of standard:basal or test:basal.

TSH bioactivity: this test is for patients having high circulating TSH levels when measured by radio-immunoassay but low circulating free T4, indicative of hypothyroidism because of defective TSH. Standards are well-

characterised euthyroid and hypothyroid serum samples of increasing biological activity.

TSAb measurements are made in thyrotoxic patients. Standards are pooled normal human serum (having a light output of ≤ 1.5 RLU), bTSH and well-
5 characterised TSAb containing sera of increasing activity.

TBAb measurements are made in the same patients as in (i), but the assay wells also contain 1mU/ml of bTSH. Standards are pooled normal sera + 1mU/ml bTSH and well-characterised TBAb containing sera of increasing activity.

10

Measurement of light output

Following the incubation period, supernatants were removed from the wells and the cells are washed twice in phosphate buffered saline. Light output was measured using a commercially-available kit from the Promega
15 Corporation, no. E1483, according to the manufacturer's instructions. This involved treating the cells in lysis buffer (Promega no. E1513), adding the Firefly luciferase reagent and measuring the light output in a luminometer.

If the cells used for the assay also express Renilla luciferase constitutively (as prepared in Example 2) to give a method of standardisation
20 of cell number/well, the Promega Dual-Luciferase system is used, no. E1910. In this case, following measurement of the Firefly luciferase as above, a reagent to quench the luminescent signal is added followed by the Renilla luciferase reagent and a second reading taken.

Example 4: Selection and Use of lulu1 Cells for Assay

25 JP09 cells were subjected, as described in Example 1, to standard calcium phosphate transfection, either with 5 μ g cAMP-luciferase and 2 μ g pBABE puro or with the puromycin resistance plasmid alone. cAMP-luciferase is 846 bp of 5' flanking region and 44 bp of exon 1 of the glycoprotein hormone α subunit promoter, which contains two cAMP
30 response elements (CREs) in tandem, linked to the firefly luciferase gene (as

described by Chatterjee *et al* in Mol Endocrin 5 100-110 (1991)). Pools of puromycin resistant cells were obtained following selection and tested, in 6 well plates, for light output in response to bovine TSH (as described in Example 3). Colonies were isolated using cloning rings.

5 (a) *Selection of lulu1*

The selected clones were cultured overnight in medium with 10% charcoal-stripped calf serum (Sigma) in place of FCS, followed by 4 hours' incubation with varying concentrations of bovine TSH. Light output was measured by luciferase reporter assay (Promega) in a Berthold luminometer. Results were
10 calculated as the ratio of light output in the presence of TSH:light output in the absence of TSH and expressed as relative light units (RLUs). Clones showing a good response to TSH were then cloned by limiting dilution and re-tested with bovine and human TSH and international TSA standard 90/672.

(b) *Determination of a Reference Range*

15 Approximately 2×10^4 lulu 1 cells were seeded in 96 well plates and switched to 100 μ l/well Ham's F12 containing 10% charcoal-stripped calf serum the day before the assay. 34 euthyroid sera from individuals negative for thyroglobulin and thyroperoxidase antibodies, and having no known history of thyroid disease were tested, in duplicate, by adding 10 μ l directly to
20 the wells and incubating at 37°C for 4 hours. Cells were assayed as described in (a) above, but using a Berthold 96 well plate luminometer. Results are expressed in RLU, as the ratio between the light output in the presence of the individual serum:light output in the absence of serum. Subsequently, the 34 sera were pooled to provide a negative control.

25 Sera from 100 treated patients with GD, 50 negative in a commercial TBII (TSH-R) assay (TRAK, BRAHMS Diagnostica, Berlin; the cut-off was 9 units, and the functional assay sensitivity and upper limit of detection were 8 and 405 TRAK units, respectively) and 50 positive, were assayed in duplicate, by adding 10 μ l serum directly to the wells. The assay was also performed on
30 20 Hashimoto's, 27 multinodular (8 toxic) goitre, 20 systemic lupus

erythematosus and 12 rheumatoid factor positive arthritis sera. All results were calculated in RLU as in (a) above.

(c) *Comparison of cAMP measured by luminescence/RIA*

44 of the GD sera described above, (35 TBII (TSH-R) positive) and the
 5 TAb standard, were also assayed in a traditional bioassay in which cAMP released into the culture medium was measured by RIA. Lulu 1 were seeded in 96 well plates and the assay was performed in 100 μ l/well NaCl-free Hank's medium, containing 2 mM IBMX and 10 μ l individual GD or pooled euthyroid serum. cAMP was measured by the cAMP [3 H] assay system (Amersham) as
 10 described by Ludgate *et al* in Exp Clin Endo 100 73-4. These results are shown in the following table (*Table 1*), in which: ^ = mean luminometer readings (n = 3) with background light emission subtracted, (SEM); and * = mean (n=2) pmoles cAMP, (SEM).

<i>TABLE 1</i>	^RLUs	*pmole cAMP
blank	98 (4)	
1 mU/ml TSH	1293 (93)	9.4 (0.8)
TSAb (90/672) 10mIU/ml	1245 (80)	15.0 (1.1)
Forskolin 10^{-5} M	1651 (43)	
euthyroid	127 (5)	2.2 (0.2)

15

Detection of TAb in Treated GD

An upper limit of <1.45 RLU was derived from the 97.5th percentile of analysis of 34 euthyroid samples (range 0.96 - 1.48 RLU). When the GD sera were assayed in physiological conditions, 66% of the TBII negative and 80%
 20 of the TBII positive sera produced >1.5 R.L.U. in the luminescent assay, which was obtained with only 4% of the various disease group control sera.

The intra-assay variation was 11.9%, calculated using the 100 GD sera and the inter-assay variation was 14.6% calculated from the 100 GD samples measured in two separate assays by paired analysis.

33 of the sera were positive in the luminescent assay, 27 by RIA, 7 were negative in both assays and 6 were positive by RIA but negative in the luminescent assay. Results using the three assays for TSH-R antibodies in the 100 GD sera are shown in the following table (*Table 2*):

TABLE 2	TBII	TSAb (lumi)	TSAb (RIA)
All treated GD	50/100	73/100	
TBII +ve GD	50/50	40/50	
TBII -ve GD	0/50	33/50	
treated GD	35/44	33/44	27/44

In the conditions employed, the luminescent bioassay of this invention performed better than the traditional RIA measurement for cAMP, perhaps since the indirect measurement, via light output, amplifies the response. The behaviour of the standard exemplifies this: in the luminescent assay, the light output was approximately 10 times that of the normal pool; while, in the RIA, it gave only a 7-fold increase.

It has also been observed that TSH responsiveness is decreased in salt-free (NaCl-free) conditions, and preliminary studies show that the luminescent assay is more sensitive for measuring TSAb but less for TSH when compared with isotonic.

CLAIMS

1. An assay method for TSH-R autoantibodies or TSH, which method comprises step:
 - 5 (a) contacting a test sample, in the presence or absence of TSH, with cells from a clone expressing human TSH-R transfected with a reporter construct comprising cDNA of both (i) a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein, and (ii) a promoter containing cyclic AMP (cAMP) response elements (CREs), whereby cAMP levels vary with expression of the reactant.
- 10 2. An assay method according to claim 1, further comprising step:
 - 15 (b) adding the corresponding substrate to cells thus contacted.
3. An assay method according to claim 2, further comprising steps:
 - (c) measuring the response in the cells exposed to the substrate; and
 - 20 (d) comparing the response from test step (c) with the response from a standard or normal sample which has undergone steps (a) to (c).
4. An assay method according to any of claims 1 to 3, in which the measurable response is a colour change, fluorescence change or emission of light.
- 25 5. An assay method according to claim 4, wherein the reactant is selected from chloramphenicol acetyl transferase (CAT), Firefly luciferase, Renilla luciferase, β -galactosidase, alkaline phosphatase, horseradish
- 30

peroxidase and green fluorescent protein.

6. An assay method according to any preceding claim, wherein the cyclic AMP response element (CRE) of the reporter construct comprises a promoter sequence or synthetic oligonucleotide which contains the CRE consensus sequence, TGACGTCA.
7. An assay method according to claim 6, wherein the cyclic AMP response element (CRE) comprises a tandem repeat of the CRE consensus sequence, TGACGTCA.
8. An assay method according to any preceding claim, wherein the promoter is that for the glycoprotein hormone alpha subunit that contains tandem cAMP response elements.
9. An assay method according to any of claims 1 to 6, wherein the promoter comprises a construct driving the CAT enzyme.
10. An assay method according to claim 3, which comprises, in step (a), the use of a luciferase cDNA driven by a promoter containing cAMP response elements; in step (b), the use of luciferin; and, in step (c), measuring the light output from the luciferinised cells.
11. An assay method according to any preceding claim, wherein the reporter construct comprises α -luciferase.
12. An assay method according to any preceding claim, wherein all reagents used therein are brought together in one or more steps; and/or wherein two or more of the steps (a) to (d) are carried out substantially simultaneously.

13. An assay method according to any preceding claim, which is carried out by manual, partly automated or fully automated means.
- 5 14. A kit for carrying out an assay according to any preceding claim.
15. A kit according to claim 14, which kit comprises:
- (a) cells from a clone expressing human TSH-R transfected with a reporter construct comprising cDNA of both a reactant, such as
10 an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein, and a promoter containing cyclic AMP (cAMP) response elements (CREs), whereby cAMP levels vary with expression of the reactant;
- 15 (b) a standard sample for the assay;
- (c) medium for culturing and/or reconstituting the cells; and
- (d) instructions for carrying out the assay.
16. A kit according to claim 15, further comprising:
- 20 (e) buffer for lysing the cells; and/or
- (f) buffer for the reporter construct, preferably luciferase buffer; and/or
- (g) corresponding substrate, preferably luciferin, in buffer; and, optionally, a luminometer.
- 25 17. A kit according to any of claims 14 to 16, wherein the reporter construct comprises the plasmid pA3luc having the glycoprotein hormone α subunit promoter introduced therein.
- 30 18. A kit according to any of claims 14 to 17, wherein the CRE-containing

sequence is sub-cloned into a commercially-available luciferase reporter system, such as pGEM-luc.

19. A kit according to any of claims 14 to 17, wherein the reporter construct
5 comprises a plurality of plasmids.
20. A kit according to any of claims 14 to 17, wherein the human TSH-R is sub-cloned into a eukaryotic expression vector.
- 10 21. A kit according to claim 20, wherein said eukaryotic expression vector is pSVL.
22. A kit according to claim 20, wherein the TSH-R is sub-cloned into a dual vector that incorporates the antibiotic resistance gene within the
15 same plasmid.
23. A kit according to claim 22, wherein the dual vector comprises pcDNAIII.
- 20 24. A kit according to any of claims 14 to 23, wherein the cells for component (a) are from clone JP09 as identified herein, which have been stably transfected within the order of 10^5 TSH-R per cell.
- 25 25. A kit according to claim 24, wherein said cells are co-transfected with both α -luciferase cDNA and a puromycin resistance encoding plasmid.
26. A kit according to any of claims 14 to 25, wherein the cells are lyophilised (freeze-dried), frozen or comprised in a gel, and provided in individual containers.

27. A kit according to any of claims 14 to 25, wherein said cells are further co-transfected to provide the assay with a method of correcting for the number of cells seeded in a well during use.
- 5 28. A kit according to claim 27, wherein said cells are further co-transfected using a Renilla luciferase plasmid.
29. An assay method or a kit according to any preceding claim for use in association with a condition or disease selected from: autoimmune
10 thyroid disease, non-autoimmune thyroid disease, autoimmunity of non-thyroid origin and polyendocrine disease.
30. An assay method or a kit according to any preceding claim for use in screening patients selected from: pregnant women, those with
15 euthyroid eye disease, and those receiving amiodarone and/or lithium.
31. An assay method or kit according to any preceding claim for measuring TSAAb or TBAAb, or for measuring autoantibodies to the TSH-R having part of its sequence modified, such as by having one or more of its
20 amino acids replaced or otherwise modified to include tags.
32. A reporter construct comprising cDNA of both a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate and a promoter containing
25 cAMP response elements, whereby increased cAMP levels vary with expression of the substrate.
33. A reporter construct according to claim 32 wherein the reactant enzyme is a luciferase.

34. A clone expressing human TSH-R transfected with a reporter construct according to claim 32 or claim 33.
35. Cells produced by a clone according to claim 34.
- 5 36. cDNA or mRNA expressing human TSH-R transfected with a reporter construct according to claim 32 or claim 33.
- 10 37. Human TSH-R transfected with a reporter construct comprising cDNA of both a reactant, such as an enzyme, capable of causing a measurable response when brought into contact with a corresponding substrate, such as a protein, and a promoter containing CRE.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 98/02904

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/68 C12N15/12 C12N5/18 C07K14/72

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KRISHNA V. ET AL.,: "Repression of the human glycoprotein hormone α -subunit gene by glucocorticoids:evidence for receptor interactions with limiting transcriptional activators" MOL. ENDOCRINOLOGY, vol. 5, no. 1, - January 1991 pages 100-110, XP002089743	32
A	see whole doc. esp. figures 4 and 5 --- -/--	8,17

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

12 January 1999

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INTERNATIONAL SEARCH REPORT

Inter: International Application No

PCT/GB 98/02904

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HIMMLER A ET AL: "FUNCTIONAL TESTING OF HUMAN DOPAMINE D1 AND D5 RECEPTORS EXPRESSED IN STABLE CAMP-RESPONSIVE LUCIFERASE REPORTER CELL LINES" JOURNAL OF RECEPTOR RESEARCH, vol. 13, no. 1/04, 1 January 1993, pages 79-94, XP000471667 see the whole document ---	1-37
Y	LUDGATE M ET AL: "USE OF THE RECOMBINANT HUMAN THYROTROPIN RECEPTOR (TSH-R) EXPRESSED IN MAMMALIAN CELL LINES TO ASSAY TSH-R AUTOANTIBODIES" MOLECULAR AND CELLULAR ENDOCRINOLOGY, vol. 73, 1 January 1990, pages R13-R18, XP000283191 see the whole document ---	1-37
A	PERSANI L. ET AL.: "Measurement of cAMP accumulation in chinese hamster ovary cells transfected with recombinant human TSH receptor (CHO-R): a new bioassay for human thyrotropin" J. ENDOCRINOL. INVEST., vol. 16, - July 1993 pages 511-519, XP002089744 see the whole document ---	1-37
A	LIBERT F ET AL: "CLONING, SEQUENCING AND EXPRESSION OF THE HUMAN THYROTROPIN (TSH) RECEPTOR EVIDENCE FOR BINDING OF AUTOANTIBODIES" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 165, no. 3, 29 December 1989, pages 1250-1255, XP000083569 see the whole document ---	1-37
T	WATSON P.F. ET AL.: "A new chemiluminescent assay for the rapid detection of thyroid stimulating antibodies in Graves' disease" CLIN. ENDOCRINOLOGY, vol. 49, - November 1998 pages 577-581, XP002089745 see the whole document -----	